A large number of toxins from sea anemone have been previously shown to modulate voltage-gated Na+ channel (Nav) function. The aim of this study was to test whether APETx2, at concentrations used to block heterotrimeric ASIC3 containing channels, affects Nav function in sensory neurons. The effect of APETx2 on Nav function was studied using the whole-cell patch-clamp technique on acutely dissociated small-diameter rat dorsal root ganglion (DRG) neurons.
In our study, APETx2 inhibited the tetrodotoxin (TTX)-resistant Nav1.8 currents of DRG neurons in a concentration-dependent manner with an IC50 of ~3 μM. TTX-sensitive currents were inhibited to a smaller extent. The observed inhibition of Nav1.8 currents is due to a rightward shift in the voltage dependence of activation, and a reduction of the maximal macroscopic conductance. In current-clamp experiments in DRG neurons the number of action potentials induced by injection of a current ramp was reduced by APETx2.
APETx2 inhibits, in addition to ASIC3, Nav1.8 channels at concentrations used in in vivo studies. The limited specificity of this toxin should be taken into account when using APETx2 as a pharmacological tool. Its dual action will be an advantage for the use of APETx2 or its derivatives as analgesic drugs.

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Tailoring μ-Conotoxin-KIIIA to Selectively Inhibit NaV1.7
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Voltage-gated sodium(NaV) channels are critical in initiating and propagating nerve impulses in a variety of tissues including, skeletal(NaV1.4) and cardiac (NaV1.5) muscle, brain(NaV1.2), and peripheral nerve(NaV1.7). Of special interest as a potential drug target is NaV1.7, which mediates propagation of pain signals from receptors to the CNS. Selective blockers of this channel could offer an important analgesic treatment. μ-Conotoxin KIIIA blocks NaV1.2, 1.4 and 1.7, expressed in mammalian cells(Kds: 5, 37, and 97nM respectively). We found two positions in KIIIA, where substitutions alter its selectivity among these channels. KIIIA-H12A shows increased potency for NaV1.7 compared with NaV1.2 and 1.4, but remains more potent against NaV1.2 than NaV1.7(Kds, μM: NaV1.2,10.8; NaV1.4,110 and NaV1.7,19. The second derivative, KIIIA-R14A, inhibits NaV1.7 more strongly than both NaV1.2 and NaV1.4 with Kds (μM): 0.5, 1.1 and 5.7 for NaVs 1.7, 1.2, and 1.4, respectively. These changes in toxin selectivity are associated with differences in the outer ring charges of these channels. The ''outer ring'' pore-vestibule residues in all NaV channels, except hNaV1.7, are EEDD. In hNaV1.7, the third aspartate is replaced by isoleucine. We have tested reciprocal mutations at this position(NaV1.4D/I and NaV1.7I/D), and these mutations account for the differences in Kd for the wild-type toxin. These channel mutants show no difference from the native channels for block by KIIIA-R14A, suggesting the interaction between the toxin/channel pair has been removed (double mutant cycle analysis yields ΔΔG=~1.7kT). We further examined these two positions by synthesizing the double mutant, KIIIA-H12Q/R14A, to examine its potency against NaV1.7. Preliminary data indicate that the double mutant(KIIIA-H12Q/R14A) inhibits NaV1.7 more strongly than either NaV1.2 or NaV1.4(Kds:23, 286, and 70 μM, respectively). Thus, interactions with this channel residue could be important in designing blockers that differentially interact with the outer ring to increase their selectively for NaV1.7.

1650-Pos Board B420
Mapping the Receptor Sites for a β-Scorpion Toxin on the Pore Module in Domain III of Voltage-Gated Sodium Channels
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Activation of voltage-gated sodium (Na_v) channels initiates and propagates action potentials in electrically excitable cells. The -scorpion toxin CssIV traps a voltage-sensor of Na_v channels in its activated state via a voltage-sensor trapping mechanism and thus shifts their voltage dependence of activation to more negative membrane potentials. The SS2-S6 linker of the pore domain in domain III (IIISS2-S6) is crucial in determining the action of CssIV upon Na_v channels. We found that five substitutions at four amino acid residues in IIISS2-S6 markedly alter voltage-sensor trapping current (I_{VST}) by a recombinant toxin derivative, $CssIV^{E15A}$. These residues are concentrated in the region between N1436 and D1445 and form a discontinuous interaction site. Three of them (E1438A, D1445A and D1445Y) markedly decrease I_{VST}, whereas the other two (N1436G and L1439A) markedly increase I_{VST}. N1436G increases binding affinity of $CssIV^{E15A}$ to Na_v channels in the resting state, whereas L1439A increases the efficacy of trapping the activated voltage-sensor by the prebound toxin. Time courses of voltage sensor trapping for the WT and mutant channels fit an allosteric kinetic model that includes a lower affinity resting state and a higher affinity activated/trapped state. Structural modeling suggests that the IIISS2-S6 is in close proximity to the IIS1-S2 and IIS3-S4 linkers in 3D space and that the bound toxin sits in a cleft formed by these three extracellular loops. Our results define the molecular map of a third interacting component of the -scorpion toxin receptor site of mammalian Na_v channels and provide new molecular details of the voltage-sensor trapping mechanism of toxin action.

1651-Pos Board B421
Crystallographic Basis for Calcium Regulation of Sodium Channels
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Voltage-gated sodium channels underlie the rapid regenerative upstroke of action potentials and are modulated by cytoplasmic calcium ions through a poorly understood mechanism. We describe the 1.35 Å crystal structure of Ca2+ bound calmodulin (Ca2+/CaM) in complex with the inactivation gate (DIII-IV linker) of the cardiac sodium channel (NaV1.5). In conjunction with isothermal titration calorimetry (ITC), we identify novel inactivation gate mutations that enhance or diminish Ca2+/CaM binding, which, in turn, sensitize or abolish Ca2+ regulation of full-length channels in electrophysiological experiments. Additional ITC experiments support a model whereby a single Ca2+/CaM bridges the C-terminus IQ motif to the DIII-IV linker via individual N- and C-lobes. The data suggest that Ca2+/CaM impedes binding of the inactivation gate to its receptor, thus biasing inactivation toward more depolarized potentials.

1652-Pos Board B422
Calmodulin Discrimination Between Voltage-Dependent Sodium Channel IQ Motifs
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Voltage-dependent sodium channels in the **Na_V1** family control action potentials in neurons and muscle. They are regulated by calmodulin (**CaM**), an essential eukaryotic calcium sensor that contains two highly homologous domains (**N** and **C**). Na_V channelopathies include epilepsy, Long QT syndrome, ventricular fibrillation, familial autism, and pain insensitivity. Some mutations are in or near an **IQ motif** (IQxxxBGxxxB, B = K, R) in the intracellular C-terminal tail of the pore-forming alpha subunit where CaM binds tightly. In the 11 residues of each Na_V IQ motif, only the Q is completely conserved. In $Na_V1.2$ (2KXW) and $Na_V1.5$ (2L53), both apo and Ca^{2+}-saturated CaM anchor to the IQ motif via **C**. However, Ca^{2+} binding to CaM changes its conformation and lowers its affinity for the IQ motif. To determine how CaM translates changes in intracellular [Ca^{2+}] into conformational work, and how it regulates multiple isoforms of Na_V, we are using NMR and fluorescence to test how CaM discriminates between IQ motifs of Na_V and how disease-causing mutations uniquely affect binding of apo and calcium-saturated **N** and **C.** To determine energetic differences in CaM binding to IQ motifs, we embedded each sequence into an auto-fluorescent biosensor (YFP-IQ-CFP) to determine their affinity for CaM, and each CaM domain individually. Biosensor quantum yield permits resolution of dissociation constants close to 1 nM from equilibrium titrations. Based on thermodynamic and structural studies of $(Ca^{2+})_4$-CaM bound to IQ motifs in the Ca_V family, a homology model is proposed for $(Ca^{2+})_4$-CaM bound to $Na_V1.2$. NIH R01 GM57001 and Carver Charitable Trust Grant 01-224.
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Ca^{2+} Dependent Inhibition of Cardiac Sodium Channel
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Cardiac sodium channels ($Na_v1.5$) are principal molecular determinants responsible for myocardial conduction and maintenance of the cardiac rhythm. Intracellular calcium can trigger dual-mode regulation of the cardiac sodium channel but the mechanisms whereby intracellular Ca^{2+} may directly modulate Na^+ channel function have yet to be identified.
Whole-cell and perforated patch clamp experiments were performed to study the effects of NVP-1 on the hNav1.5. In the automated patch clamp Nav1.5 assay on the IonWorks Quattro system (perforated patch clamp mode), NVP-1 was inactive, producing only 13% inhibition of the Na^+ current (I_{Na}) at 50 μM, while in conventional patch clamp experiments (in whole-cell patch clamp mode) it blocked the channel with an IC_{50} value of 5.45 μM. In conventional patch clamp experiments intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was very